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Practitioner's Docket No. U 013220-5

Optional Customer No. Bar Code

00140
PATENT TRADEMARK OFFICE

CHAPTER II

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US) (ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

INTERNATIONAL APPLICATION NO. CLAIMED	INTERNATIONAL FILING DATE	PRIORITY DATE
PCT/IL99/00396	19 JULY 1999	20 JULY 1998

TITLE OF INVENTION

CONTROLLING STARCH SYNTHESIS

APPLICANT(S)

1. ARTHUR SCHAFFER
2. IIAN LEVIN
3. MARINA PETREIKOV
4. MOSHE BAR

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

NOTE: The completion of those filing requirements that can be made at a time later than 30 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 USC 371(d). The filing

CERTIFICATION UNDER 37 C.F.R. 1.10*
*(Express Mail label number is **mandatory**.)*
(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date JANUARY 19, 2001, in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number EL 728210649 US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

BARBARA D. SANTIAGO

(type or print name of person mailing paper)

Barbara D. Santiago

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

***WARNING:** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 C.F.R. §1.491 which states: "An international application enters the national state when the applicant has filed the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495."

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. §1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 C.F.R. §1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 C.F.R. § 1.494(f).

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

   a. [X] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

   b. [X] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

i. [X] A check in the amount of $565.00 to cover the above fees is enclosed.
ii. [ ] Please charge Account No. _____ in the amount of $ _____.
A duplicate copy of this sheet is enclosed.

**WARNING. *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. [X] A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: *Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.*

a. [ ] is transmitted herewith.
b. [ ] is not required, as the application was filed with the United States Receiving Office.
c. [X] has been transmitted
i. [X] by the International Bureau.
Date of mailing of the application (from form PCT/IB/308): _____.
ii. [ ] by applicant on _____.
Date

4. [X] A translation of the International application into the English language (35 U.S.C. 371(c)(2)):
a. [X] is transmitted herewith.
b. [ ] is not required as the application was filed in English.
c. [ ] was previously transmitted by applicant on _____.
Date
d. [ ] will follow.

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. [ ] are transmitted herewith.
b. [ ] have been transmitted
   i. [ ] by the International Bureau.
   Date of mailing of the amendment (from form PCT/IB/308): _____.
   ii. [ ] by applicant on _____.
   Date
c. [X] have not been transmitted as
   i. [X] applicant chose not to make amendments under PCT Article 19.
   Date of mailing of Search Report (from form PCT/ISA/210): 14 JUNE 2000.
   ii. [ ] the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):
a. [ ] is transmitted herewith.
b. [ ] is not required as the amendments were made in the English language.
c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)
[X] is transmitted herewith.
[ ] is not required as the application was filed with the United States Receiving Office.

8. [ ] Annex(es) to the international preliminary examination report
a. [ ] is/are transmitted herewith.
b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [ ] A translation of the annexes to the international preliminary examination report
a. [ ] is transmitted herewith.
b. [ ] is not required as the annexes are in the English language.

10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115

a. [ ] was previously submitted by applicant on _____.
Date

b. [ ] is submitted herewith, and such oath or declaration
i. [ ] is attached to the application.
ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.

c. [X] will follow.

Other document(s) or information included:

11. [X] An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. [X] is transmitted herewith.
b. [ ] has been transmitted by the International Bureau.
Date of mailing (from form PCT/IB/308): _____.
c. [ ] is not required, as the application was searched by the United States International Searching Authority.
d. [ ] will be transmitted promptly upon request.
e. [ ] has been submitted by applicant on _____.
Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:

a. [ ] is transmitted herewith.
Also transmitted herewith is/are:
[ ] Form PTO-1449 (PTO/SB/08A and 08B).
[ ] Copies of citations listed.
b. [X] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
c. [ ] was previously submitted by applicant on _____.
Date

13. [ ] An assignment document is transmitted herewith for recording.

A separate [ ] "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ] FORM PTO 1595 is also attached.

_____
_____
_____

14. [X] Additional documents:
a. [ ] Copy of request (PCT/RO/101)
b. [X] International Publication No. WO 00/05390
i. [X] Specification, claims and drawing
ii. [ ] Front page only
c. [ ] Preliminary amendment (37 C.F.R. § 1.121)
d. [X] Other

PCT/IPEA/416

15. [X] The above checked items are being transmitted
a. [X] before 30 months from any claimed priority date.
b. [ ] after 30 months.

16. [ ] Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on
_____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

NOTE: "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 12-0425.

[X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

[ ] 37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must

only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

[X] 37 C.F.R. 1.17 (application processing fees)
[X] 37 C.F.R. 1.17(a)(1)-(5)(extension fees pursuant to § 1.136(a).
[X] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

[ ] 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF PRACTITIONER

Reg. No.: 20302

JULIAN H. COHEN
(type or print name of practitioner)

Tel. No.: (212)708-1887

LADAS & PARRY
P.O. Address

Customer No.: 00140

26 WEST 61ST STREET
NEW YORK, NEW YORK 10023

Applicant or Patentee: ARTHU[redacted]SCHAFFER, et al Attorneys [redacted]ket No. U 013220-5
Serial or Patent No.. 09/744,085
Filed or Issued:
For: CONTROLLING STARCH SYNTHESIS

#6

VERIFIED STATEMENT [DECLARATION] CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) AND 1.27 (c)) – SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below;
[ ] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: **STATE OF ISRAEL-MINISTRY OF AGRICULTURE**
ADDRESS OF CONCERN: **Volcani Research Center, P.O. Box 6, 50250 Beit Dagan, Israel**

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9 (d), for purpose of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled **ALKALINE ALPHA-GALACTOSIDASE**, by the inventor's **Arthur A. SCHAFFER, Gao ZHIFANG** described in

[ ] the specification filed herewith
[x] application serial no. 09/744,085, filed January 19, 2001
[ ] patent no. _____, issued _____

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(c).
*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averting to their status as small entities. (37CFR 1.27)

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR {1.28}(b)).
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING * David Levy
TITLE OF PERSON OTHER THAN OWNER * Director
ADDRESS OF PERSON SIGNING * Volcani Center

SIGNATURE * David Levy DATE *

#9/a

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Arthur SCHAFFER, et al

Serial No.: 09/744,085 Group No.:

Filed: January 19, 2001 Examiner:

For: CONTROLLING STARCH SYNTHESIS

Attorney Docket No.: U-013220-5

Commissioner Patents and Trademarks
Washington, DC 20231

AMENDMENT

Sir:

In response to the Official Action of April 17, 2001, please amend the application as follows:

IN THE SPECIFICATION:

Page 12, delete lines 20 - end and rewrite as follows:

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, DC 20231

CLIFFORD J. MASS
Type or print name of person mailing paper)

Date: June 18, 2001

(Signature of person mailing paper)

ADPGPPase Subunit	Forward primer	Reverse primer	Restriction endonuclease
Large (LS1)	GTTCATTTGGGGA GAGTGAGCAC (Seq. ID No. 1)	GGGCAGCAGAAT TGTACTGTGTC (Seq. ID No. 2)	Hinf I
Large (LS2)	CTATTGGTGGTTG TTACCGGGT (Seq. ID No. 3)	CACTGTTCCAATA TCCTCCCAG (Seq. ID No. 4)	Hinf I
Large (LS3)	GCATATTGCTCGT GCGTACAAC (Seq. ID No. 5)	CTTTTCGCTGAAG GACATGACC (Seq. ID No. 6)	-
Small	TTTCGTCTTCTCA TCTCGCCGGA (Seq. ID No. 7)	GGCGATTTAGAG AGGCAGAGTTG (Seq. ID No. 8)	RsaI

Page 13, please delete the last paragraph and rewrite as follows:

Table 6 is the nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L.hirsutum* (Seq. ID No. 9). Table 7 is the derived amino acid sequence for ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum* (Seq. ID No. 10).

Page 14, please delete all paragraphs and rewrite as follows:

Table 6: Nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum* (Seq. ID No. 9).

1 ATGAAATCGA CGGTTCATTT GGGGAGAGTG AGCACTGGTG GCTTTAACAA
51 TGGAGAGAAG GAGATTTTTG GGGAGAAGAT GAGAGGGAGT TTGAACAACA
101 ATCTCAGGAT TAATCAGTTG TCGAAAAGTT TGAAACTTGA GAAGAAGGAG
151 AAGAAGATTA AACCTGGGGT TGCTTACTCT GTGATCACTA CTGAAAATGA
201 CACAGAGACT GTGTTCGTAG ATATGCCACG TCTTGAGAGA CGCCGGGCAA
251 ATCCCAAGGA TGTGGCTGCA GTCATATTAG GAGGAGGCGA AGGGACCAAG
301 TTATTCCCAC TTACAAGTAG AACTGCAACC CCTGCTGTTC CGGTTGGAGG
351 ATGCTACAGG CTCATAGACA TCCCGATGAG CAACTGTATC AACAGTGCTA
401 TTAACAAGAT TTTTGTGCTG ACACAGTACA ATTCTGCTGC CCTGAATCGT
451 CACATTGCTC GAACGTATTT TGGCAATGGT GTGAGCTTTG GAGATGGATT
501 TGTCGAGGTA CTAGCTGCAA CTCAGACACC TGGGGAAGCA GGAAAAAAAT
551 GGTTTCAAGG AACAGCAGAT GCTGTCAGAA AATTTATATG GGTTTTTGAG
601 GACGCTAAGA ACAAGAATAT TGAAAATATC CTTGTATTAT CTGGGGATCA
651 TCTTTATAGG ATGGATTATA TGGAGTTGGT GCAGAACCAT ATTGACAGAA
701 ATGCTGATAT TACTCTTTCA TGTGCACCAG CTGAGGACAG CCGAGCATCA
751 GATTTTGGGC TGGTCAAGAT TGACAGCAGA GGCAGAGTTG TCCAGTTTGC
801 TGAAAAACCA AAAGGTTTTG AGCTTAAAGC AATGCAAGTA GATACTACTC
851 TTGTTGGATT ATCTCCACAA GATGCGAAGA AATCCCCTTA TATTGCTTCA
901 ATGGGAGTTT ATGTTTTCAA GACAGATGTA TTGCTGAAGC TCTTGAAATG
951 GAGCTACCCC ACTTCTAATG ATTTTGGCTC TGAAATTATA CCAGCAGCTA
1001 TTGATGATTA CAATGTCCAA GCATACATTT TCAAAGACTA TTGGGAGGAC
1051 ATTGGAACAA TTAAATCTTT CTATAATGCT AGCTTGGCGC TCACACAAGA
1101 GTTTCCAGAG TTCCAATTTT ATGATCCAAA AACACCTTTT TACACATCTC
1151 CTAGGTTCCT TCCACCAACC AAGATAGACA ATTGCAAGAT TAAGGATGCC
1201 ATAATTTCTC ATGGATGTTT CTTGCGAGAT TGCTCTGTGG AACACTCCAT
1251 AGTGGGTGAA AGATCACGCT TAGACTGTGG TGTTGAACTG AAGGATACTT
1301 TCATGATGGG AGCAGACTAC TACCAAACAG AATCTGAGAT TGCCTCCCTG
1351 TTAGCAGAGG GGAAAGTACC GATTGGGATT GGGGAAAATA
CAAAAATAAG
1401 GAAATGTATC ATTGACAAGA ACGCAAAGAT AGGAAAAAAT GTTTCAATCA
1451 TTAATAAAGA TGGTGTTCAA GAGGCAGACC GACCAGAGGA AGGATTCTAC
1501 ATACGATCAG GGATAACCAT TATATCAGAG AAAGCCACAA TTAGAGATGG
1551 AACAGTTATA TGA

Table 7: Derived amino acid sequence for ADPGPPase LS1 from *L. hirsutum* (Seq. ID No. 10).

MKSTVHLGRVSTGGFNNGEKEIFGEKMRGSLNNNLRINQL
SKSLKLEKKEKKIKPGVAYSVITTENDTETVFVDMPRLERRRAN
PKDVAAVILGGGEGTKLFPLTSRTATPAVPVGGCYRLIDIPMSNC
INSAINKIFVLTQYNSAALNRHIARTYFGNGVSFGDGFVEVLAAT
QTPGEAGKKWFQGTADAVRKFIWVFEDAKNKNIENILVLSGDHL
YRMDYMELVQNHIDRNADITLSCAPAEDSRASDFGLVKIDSRGR
VVQFAEKPKGFELKAMQVDTTLVGLSPQDAKKSPYIASMGVYV
FKTDVLLKLLKWSYPTSNDFGSEIIPAAIDDYNVQAYIFKDYWED
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KDAIISHGCFLRDCSVEHSIVGERSRLDCGVELKDTFMMGADYY
QTESEIASLLAEGKVPIGIGENTKIRKCIIDKNAKIGKNVSIINKDG

IN THE ABSTRACT:

Please insert the following Abstract on a separate page:

-- **ABSTRACT OF THE DISCLOSURE**

A method for controlling starch synthesis in tomatoes including providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes, and selecting individuals of the population that each contain an allele of a gene that increases starch synthesis, the gene originating from the *Lycopersicon* spp.--

After the Abstract insert the following Sequence Listing on a separate page:

SEQUENCE LISTING

<110> Schaffer, Arthur
Levin, Ilan
Petreikov, Marina
Bar, Moshe

<120> Controlling Starch Synthesis

<130> U-013220-5

<140> US 09/744,085
<141> 2001-03-26

<150> PCT/IL99/00396
<151> 1999-07-19

<150> IL 125425
<151> 1998-07-20

<160> 10

<170> PatentIn Ver. 2.1

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1 5 10 15

aat gga gag aag gag att ttt ggg gag aag atg aga ggg agt ttg aac 96

```
Asn Gly Glu Lys Glu Ile Phe Gly Glu Lys Met Arg Gly Ser Leu Asn
                20                  25                  30

aac aat ctc agg att aat cag ttg tcg aaa agt ttg aaa ctt gag aag   144
Asn Asn Leu Arg Ile Asn Gln Leu Ser Lys Ser Leu Lys Leu Glu Lys
          35                  40                  45

aag gag aag aag att aaa cct ggg gtt gct tac tct gtg atc act act   192
Lys Glu Lys Lys Ile Lys Pro Gly Val Ala Tyr Ser Val Ile Thr Thr
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gaa aat gac aca gag act gtg ttc gta gat atg cca cgt ctt gag aga   240
Glu Asn Asp Thr Glu Thr Val Phe Val Asp Met Pro Arg Leu Glu Arg
 65                  70                  75                  80

cgc cgg gca aat ccc aag gat gtg gct gca gtc ata tta gga gga ggc   288
Arg Arg Ala Asn Pro Lys Asp Val Ala Ala Val Ile Leu Gly Gly Gly
                85                  90                  95

gaa ggg acc aag tta ttc cca ctt aca agt aga act gca acc cct gct   336
Glu Gly Thr Lys Leu Phe Pro Leu Thr Ser Arg Thr Ala Thr Pro Ala
           100                 105                 110

gtt ccg gtt gga gga tgc tac agg ctc ata gac atc ccg atg agc aac   384
Val Pro Val Gly Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn
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Cys Ile Asn Ser Ala Ile Asn Lys Ile Phe Val Leu Thr Gln Tyr Asn
  130                 135                 140

tct gct gcc ctg aat cgt cac att gct cga acg tat ttt ggc aat ggt   480
Ser Ala Ala Leu Asn Arg His Ile Ala Arg Thr Tyr Phe Gly Asn Gly
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gtg agc ttt gga gat gga ttt gtc gag gta cta gct gca act cag aca   528
Val Ser Phe Gly Asp Gly Phe Val Glu Val Leu Ala Ala Thr Gln Thr
                165                 170                 175

cct ggg gaa gca gga aaa aaa tgg ttt caa gga aca gca gat gct gtc   576
Pro Gly Glu Ala Gly Lys Lys Trp Phe Gln Gly Thr Ala Asp Ala Val
           180                 185                 190

aga aaa ttt ata tgg gtt ttt gag gac gct aag aac aag aat att gaa   624
Arg Lys Phe Ile Trp Val Phe Glu Asp Ala Lys Asn Lys Asn Ile Glu
      195                 200                 205

aat atc ctt gta tta tct ggg gat cat ctt tat agg atg gat tat atg   672
Asn Ile Leu Val Leu Ser Gly Asp His Leu Tyr Arg Met Asp Tyr Met
  210                 215                 220

gag ttg gtg cag aac cat att gac aga aat gct gat att act ctt tca   720
Glu Leu Val Gln Asn His Ile Asp Arg Asn Ala Asp Ile Thr Leu Ser
225                 230                 235                 240
```

```
tgt gca cca gct gag gac agc cga gca tca gat ttt ggg ctg gtc aag   768
Cys Ala Pro Ala Glu Asp Ser Arg Ala Ser Asp Phe Gly Leu Val Lys
                245                 250                 255

att gac agc aga ggc aga gtt gtc cag ttt gct gaa aaa cca aaa ggt   816
Ile Asp Ser Arg Gly Arg Val Val Gln Phe Ala Glu Lys Pro Lys Gly
            260                 265                 270

ttt gag ctt aaa gca atg caa gta gat act act ctt gtt gga tta tct   864
Phe Glu Leu Lys Ala Met Gln Val Asp Thr Thr Leu Val Gly Leu Ser
        275                 280                 285

cca caa gat gcg aag aaa tcc cct tat att gct tca atg gga gtt tat   912
Pro Gln Asp Ala Lys Lys Ser Pro Tyr Ile Ala Ser Met Gly Val Tyr
    290                 295                 300

gtt ttc aag aca gat gta ttg ctg aag ctc ttg aaa tgg agc tac ccc   960
Val Phe Lys Thr Asp Val Leu Leu Lys Leu Leu Lys Trp Ser Tyr Pro
305                 310                 315                 320

act tct aat gat ttt ggc tct gaa att ata cca gca gct att gat gat   1008
Thr Ser Asn Asp Phe Gly Ser Glu Ile Ile Pro Ala Ala Ile Asp Asp
                325                 330                 335

tac aat gtc caa gca tac att ttc aaa gac tat tgg gag gac att gga   1056
Tyr Asn Val Gln Ala Tyr Ile Phe Lys Asp Tyr Trp Glu Asp Ile Gly
            340                 345                 350

aca att aaa tct ttc tat aat gct agc ttg gcg ctc aca caa gag ttt   1104
Thr Ile Lys Ser Phe Tyr Asn Ala Ser Leu Ala Leu Thr Gln Glu Phe
        355                 360                 365

cca gag ttc caa ttt tat gat cca aaa aca cct ttt tac aca tct cct   1152
Pro Glu Phe Gln Phe Tyr Asp Pro Lys Thr Pro Phe Tyr Thr Ser Pro
    370                 375                 380

agg ttc ctt cca cca acc aag ata gac aat tgc aag att aag gat gcc   1200
Arg Phe Leu Pro Pro Thr Lys Ile Asp Asn Cys Lys Ile Lys Asp Ala
385                 390                 395                 400

ata att tct cat gga tgt ttc ttg cga gat tgc tct gtg gaa cac tcc   1248
Ile Ile Ser His Gly Cys Phe Leu Arg Asp Cys Ser Val Glu His Ser
                405                 410                 415

ata gtg ggt gaa aga tca cgc tta gac tgt ggt gtt gaa ctg aag gat   1296
Ile Val Gly Glu Arg Ser Arg Leu Asp Cys Gly Val Glu Leu Lys Asp
            420                 425                 430

act ttc atg atg gga gca gac tac tac caa aca gaa tct gag att gcc   1344
Thr Phe Met Met Gly Ala Asp Tyr Tyr Gln Thr Glu Ser Glu Ile Ala
        435                 440                 445

tcc ctg tta gca gag ggg aaa gta ccg att ggg att ggg gaa aat aca   1392
Ser Leu Leu Ala Glu Gly Lys Val Pro Ile Gly Ile Gly Glu Asn Thr
    450                 455                 460
```

```
aaa ata agg aaa tgt atc att gac aag aac gca aag ata gga aaa aat   1440
Lys Ile Arg Lys Cys Ile Ile Asp Lys Asn Ala Lys Ile Gly Lys Asn
465                 470                 475                 480

gtt tca atc att aat aaa gat ggt gtt caa gag gca gac cga cca gag   1488
Val Ser Ile Ile Asn Lys Asp Gly Val Gln Glu Ala Asp Arg Pro Glu
                485                 490                 495

gaa gga ttc tac ata cga tca ggg ata acc att ata tca gag aaa gcc   1536
Glu Gly Phe Tyr Ile Arg Ser Gly Ile Thr Ile Ile Ser Glu Lys Ala
            500                 505                 510

aca att aga gat gga aca gtt ata tga                              1563
Thr Ile Arg Asp Gly Thr Val Ile
        515                 520
```

<210> 10
<211> 520
<212> PRT
<213> Lycopersicon hirsutum

<400> 10

```
Met Lys Ser Thr Val His Leu Gly Arg Val Ser Thr Gly Gly Phe Asn
  1               5                  10                  15

Asn Gly Glu Lys Glu Ile Phe Gly Glu Lys Met Arg Gly Ser Leu Asn
            20                  25                  30

Asn Asn Leu Arg Ile Asn Gln Leu Ser Lys Ser Leu Lys Leu Glu Lys
        35                  40                  45

Lys Glu Lys Lys Ile Lys Pro Gly Val Ala Tyr Ser Val Ile Thr Thr
    50                  55                  60

Glu Asn Asp Thr Glu Thr Val Phe Val Asp Met Pro Arg Leu Glu Arg
65                  70                  75                  80

Arg Arg Ala Asn Pro Lys Asp Val Ala Ala Val Ile Leu Gly Gly Gly
                85                  90                  95

Glu Gly Thr Lys Leu Phe Pro Leu Thr Ser Arg Thr Ala Thr Pro Ala
            100                 105                 110

Val Pro Val Gly Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn
        115                 120                 125

Cys Ile Asn Ser Ala Ile Asn Lys Ile Phe Val Leu Thr Gln Tyr Asn
    130                 135                 140

Ser Ala Ala Leu Asn Arg His Ile Ala Arg Thr Tyr Phe Gly Asn Gly
145                 150                 155                 160

Val Ser Phe Gly Asp Gly Phe Val Glu Val Leu Ala Ala Thr Gln Thr
                165                 170                 175
```

Pro Gly Glu Ala Gly Lys Lys Trp Phe Gln Gly Thr Ala Asp Ala Val
180 185 190

Arg Lys Phe Ile Trp Val Phe Glu Asp Ala Lys Asn Lys Asn Ile Glu
195 200 205

Asn Ile Leu Val Leu Ser Gly Asp His Leu Tyr Arg Met Asp Tyr Met
210 215 220

Glu Leu Val Gln Asn His Ile Asp Arg Asn Ala Asp Ile Thr Leu Ser
225 230 235 240

Cys Ala Pro Ala Glu Asp Ser Arg Ala Ser Asp Phe Gly Leu Val Lys
245 250 255

Ile Asp Ser Arg Gly Arg Val Val Gln Phe Ala Glu Lys Pro Lys Gly
260 265 270

Phe Glu Leu Lys Ala Met Gln Val Asp Thr Thr Leu Val Gly Leu Ser
275 280 285

Pro Gln Asp Ala Lys Lys Ser Pro Tyr Ile Ala Ser Met Gly Val Tyr
290 295 300

Val Phe Lys Thr Asp Val Leu Leu Lys Leu Leu Lys Trp Ser Tyr Pro
305 310 315 320

Thr Ser Asn Asp Phe Gly Ser Glu Ile Ile Pro Ala Ala Ile Asp Asp
325 330 335

Tyr Asn Val Gln Ala Tyr Ile Phe Lys Asp Tyr Trp Glu Asp Ile Gly
340 345 350

Thr Ile Lys Ser Phe Tyr Asn Ala Ser Leu Ala Leu Thr Gln Glu Phe
355 360 365

Pro Glu Phe Gln Phe Tyr Asp Pro Lys Thr Pro Phe Tyr Thr Ser Pro
370 375 380

Arg Phe Leu Pro Pro Thr Lys Ile Asp Asn Cys Lys Ile Lys Asp Ala
385 390 395 400

Ile Ile Ser His Gly Cys Phe Leu Arg Asp Cys Ser Val Glu His Ser
405 410 415

Ile Val Gly Glu Arg Ser Arg Leu Asp Cys Gly Val Glu Leu Lys Asp
420 425 430

Thr Phe Met Met Gly Ala Asp Tyr Tyr Gln Thr Glu Ser Glu Ile Ala
435 440 445

Ser Leu Leu Ala Glu Gly Lys Val Pro Ile Gly Ile Gly Glu Asn Thr
450 455 460

Lys Ile Arg Lys Cys Ile Ile Asp Lys Asn Ala Lys Ile Gly Lys Asn

```
465                 470                 475                 480

Val Ser Ile Ile Asn Lys Asp Gly Val Gln Glu Ala Asp Arg Pro Glu
                    485                 490                 495

Glu Gly Phe Tyr Ile Arg Ser Gly Ile Thr Ile Ile Ser Glu Lys Ala
                500                 505                 510

Thr Ile Arg Asp Gly Thr Val Ile
            515                 520
```

IN THE CLAIMS:

Please cancel claims 29 and 30 and replace with the following new claims:

31. (New) A gene that controls sucrose-starch metabolism comprising a nucleotide sequence comprising SEQ ID NO:9.

32. (New) A protein that controls sucrose-starch metabolism comprising a derived amino acid sequence comprising SEQ ID NO:10.

REMARKS

The above amendatory action has been taken in conjunction with Applicants' submission of a computer readable form copy and a paper copy of the Sequence Listing to comply with the requirements of 37 CFR 1.821 - 1.825. A marked up copy of the specification to locate the amendments thereto is attached.

Applicants submit herewith a statement that the contents of the paper copy and the computer readable copy are the same and include no new matter. In this latter connection, Applicants note that an obvious error in SEQ ID NO.9 has been rectified by adding a single nucleotide "G" at position 41. This correction does not constitute "new matter" in accordance with the provisions of MPEP Section 2163.07 (II). (An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of error in the specification, but also the appropriate correction. *In re Oda*, 443 F.2d

1200, 170 USPQ 260 (CCPA 1971).").

The existence of the error in the specification and the appropriate correction would have been recognized by one skilled in the art from a comparison of Tables 6 and 7 on page 14 of the specification as filed. The nucleotide sequence of Table 6 is the nucleotide sequence of ADPGPPase LS1 from *L. hirsutum*. The amino acid sequence of Table 7 is the *derived* amino acid sequence of ADPGPPase LS1 from *L. hirsutum*, as stated, for example, on pages 13 and 14 of the specification as filed. The first 13 amino acids of the derived amino acid sequence in original Table 7 are the amino acids that are formed from the codons represented by the nucleotides at positions 1 - 39 of original Table 6. However, in order for amino acid 14 of the amino acid sequence of Table 7 to be "G" (glycine), the codon encoding the amino acid would have to be "GGU", "GGC", "GGA" or "GGG" (see Table of Codons submitted herewith). Since a review of Table 7 shows that the nucleotides are arranged in groups of ten (10) and that the group beginning with the nucleotide at position 41 contains only nine (9), it would be clear to one skilled in the art that this group is missing a nucleotide. It would also be clear that the missing nucleotide would have to be a "G" (guanine) for there to be correspondence between the codon at positions 40 - 42 of the nucleotide sequence of Table 6 and the amino acid at position 14 of the amino acid sequence of Table 7. Indeed, it is impossible to derive the amino acid sequence of Table 7 from the nucleotide sequence of Table 6 without the "G" at position 41. Furthermore, in the absence of the "G" at position 41, the nucleotide sequence of SEQ ID No. 9 is a probably meaningless truncated 26 amino acid protein.

By this amendatory action, Applicants have complied with all applicable requirements in the aforementioned Official Action. An early examination of this application on its merits is

respectfully requested.

Respectfully submitted,

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c:\docs\13220amd.jun

Table 3-6 The Genetic Code

First Position	Second Position: U	Second Position: C	Second Position: A	Second Position: G	Third Position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA *Stop*	UGA *Stop*	A
	UUG Leu	UCG Ser	UAG *Stop*	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Health in Bethesda, Maryland, observed, also in 1961, that the addition of the synthetic polynucleotide poly U (UUUUU . . .) to a cell-free system capable of making proteins leads to the synthesis of polypeptide chains containing only the amino acid phenylalanine. The nucleotide groups UUU thus must specify phenylalanine. Use of increasingly more complex, defined polynucleotides as synthetic messenger RNAs rapidly led to the identification of more and more codons. Particularly important in completing the code was the use of polynucleotides like AGUAGU, put together by the Indian organic chemist H. G. Khorana, then working in Madison, Wisconsin. Completion of the code in 1966 revealed that 61 out of the 64 possible permuted groups corresponded to amino acids, with most amino acids being coded by more than one nucleotide triplet (Table 3-6).

Start and Stop Signals Are Also Encoded Within DNA[56–59]

Initially, it was guessed that translation of an mRNA molecule would commence at one end and finish when the entire mRNA message had been read into amino acid sequences. But, in fact, translation both starts and stops at internal positions. Thus, signals must be present within DNA (and its mRNA products) to initiate and terminate translation. First to be worked out were the stop signals. Three separate codons (UAA, UAG, and UGA), first known as **nonsense codons**, do not correspond to any amino acids but instead serve as chain-termi-

CONTROLLING STARCH SYNTHESIS

FIELD OF THE INVENTION

The present invention relates to a method of breeding tomatoes with increased starch content in the young fruit and subsequently increased soluble solids content in the mature fruit. In addition, it relates to the use of genes that increase starch in the tomato.

BACKGROUND OF THE INVENTION

The solids content of ripe tomato fruit is a major determinant of its quality. Increasing the soluble solids (largely sugars and organic acids) content and thereby improving the value of industry tomatoes and the taste of fresh market tomatoes have been the goal of research projects for many years. Several approaches to improving solids levels have been taken, encompassing both agrotechnical and genetic manipulations.

Soluble solids content of tomato fruit are primarily comprised of sugars, organic acids and salts. Collectively the soluble solids content is a major determinant of fruit quality, both for industry use and for fresh market consumption Approximately half of the soluble solids content is contributed by the sugar fraction which, in all standard cultivars of *Lycopersicon esculentum*, consists of the monosaccharide reducing sugars glucose and fructose in approximately equimolar concentrations.

Several strategies to increase sugar concentration in ripe tomato fruit have been explored. Genetic manipulations include the transfer of undefined traits of high soluble solids from wild species of Lycopersicon (Rick C.M. 1974. Hilgardia 42:493-510; and Hewitt J.D., Dinar M. and Stevens M.A. 1982. J. Am. Soc. Hort. Sci. 107:896-900) and more recently the transfer of the genetic trait of sucrose accumulation from the wild *Lycopersicon chmielewskii* (Yelle S., Hewitt J.D., Robinson N.L., Damon N.S. and Bennett A.B 1988 Pl. Physiol. 87:737-740; and Yelle S., Chetelat R.T., Dorais M., Deverna J.W. and Bennett A.B. 1991. Pl. Physiol. 95:1026-1035.) and *L. hirsutum* (Miron D. and Schaffer A.A. 1991. Pl. Physiol. 95:623-627), as well as the transfer of the genetic trait of high fructose to glucose ratio in the mature fruit, from *L. hirsutum* (US Patent Application 08/530,216, the disclosure of which is incorporated herein by reference). The latter approach was made possible by the study of the components of carbohydrate metabolism in developing tomato fruit tissue with the purpose of identifying biochemical steps whose modification may lead to increased soluble carbohydrate content in the fruit (Yelle et al., 1988, 1991; Miron and Schaffer, 1991). Once identified, these biochemical processes could then be targeted for modification by classical genetic means, assisted by selection for the genotypic biochemical trait, or by molecular genetic strategies.

The young, developing tomato fruit is characterized by a transient starch accumulation which can contribute over 25% of the dry weight of the fruit tissue. Starch concentration begins to increase within days after anthesis and reaches peak concentrations before the mature green stage (Schaffer, A.A. and Petreikov, M. 1997a. Plant Physiology 113:739-746). At the mature stage this starch is practically absent in the tomato fruit tissue. It has been hypothesized that the transiently accumulated starch serves as a reservoir of carbohydrate for the later accumulation of soluble sugars in the mature fruit (Dinar M. and Stevens M.A. 1981. J. Am. Soc. Hort. Sci. 106.415-418). Dinar and Stevens laid the groundwork for this hypothesis in their study comparing seven genotypes of tomato whose total soluble solids (TSS) values in the ripe fruit spanned the spectrum from 4.6 to 6.3 °Brix. They found that TSS values in ripe fruit were positively correlated with starch content in young, immature fruit and proposed that the products of starch hydrolysis contribute to the accumulation of soluble sugars

The tomato plant translocates photosynthate to the fruit in the form of sucrose (Walker L.J. and Ho L.C. 1977 Ann. Bot. 41:813-823) and therefore, the temporal accumulation of starch will presumably be determined by temporal changes in the activities of key enzymes involved in sucrose to starch metabolism. The enzymatic pathway of starch synthesis in young tomato fruit has been studied and described (Schaffer, A A. and Petreikov, M. 1997a. Plant Physiology 113 739-746; Schaffer, A.A. and Petreikov, M. 1997b. Physiologia Plantarum 101:800-806). Four enzymes were identified that potentially limit starch accumulation in these fruit, based on their absolute activities, as well as on the developmental changes in their activities which correlate temporally with the developmental changes in starch levels. These enzymes include those that catalyze the initial steps of sucrose metabolism in the young fruit (sucrose synthase, E.C 2.4.1.13, and fructokinase, E.C. 2 7.1 4) as well as the latter steps of starch synthesis (ADP-glucose pyrophosphorylase, E.C. 2 7.7.27, and starch synthase, E.C., 2.4.1.21). In addition, Schaffer and Petreikov have shown that starch accumulation is tissue specific, localized primarily in the columella and inner pericarp tissues, and suggested that relative contributions of these tissues to fruit bulk could impact on fruit starch content.

Research has clearly shown that one of the above mentioned enzymes, ADPGPPase (ADP-glucose pyrophosphorylase), may be limiting to starch synthesis in tomato fruit, as well as in other starch accumulating tissues, such as potato tubers. In Stark D.M , Barry G.F., and Kishore G.M 1996. Ann. NY Cad Sci 792:26-36, transgenic tomato plants and potato plants were developed with a bacterial mutant form of ADPGPPase (*E. coli*, GlgC16, a glycogen overproducer). Transgenic tomatoes showed a higher starch content in the immature fruit and

an increased sugar content in the mature fruit. Transgenic potato tubers with the same bacterial gene construct also showed an increase in starch content. Reciprocally, inhibition of ADPGPPase activity decreased the starch content of transgenic potato tubers, further indicating the importance of ADPGPPase in controlling starch accumulation.

The use of a gene for ADPGPPase of bacterial origin requires molecular genetic manipulations in order for the gene to function in eucaryotic plant tissue. For example, it requires that an artificial gene construct be developed that will encode a fusion polypeptide containing a specific amino terminal transit peptide, not present in the procaryotic gene, as well as other DNA sequence additions that will cause in plant cells transcriptional termination, and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence. In comparison, the use of a plant gene for similar transformations does not require these manipulations.

In addition, the development of plants with increased or modified activity of these enzymes, based on the natural transfer through classical breeding techniques of naturally occurring alleles of these genes, can benefit from a number of advantages. For example, classical breeding techniques lead to the positioning of the desired allele in the natural position of the gene of interest, leading to genetic stability and obviating the unpredictable "position" effects characteristic of the development of transgenic organisms. In addition, with respect to consumer preferences, there are obvious advantages of a naturally derived commercial product such as a tomato fruit, compared to a transgenically derived tomato fruit.

With respect to fructokinase, two genes from tomato fruit have been identified, cloned and sequenced (Kanayama, Y. et al. 1997 Plant Physiology 113 1379-1384) One of these genes, FK2, is particularly involved in the metabolic pathway associated with starch synthesis (Kanayama et al. 1998. Plant Physiology 117.85-90). Similarly, the gene for sucrose synthase from tomato fruit has been cloned and sequenced (Wang, F., et al. Plant Physiology 103.1463-1464,) and has been shown to be the gene for sucrose synthase of sink tissue (Fu, H. and Park, W.D. Plant Cell 7·1369-1385).

With respect to ADPGPPase, the enzyme functions in higher plants as a heterotetramer, comprised of two large and two small subunits (Preiss, J. and Sivak, M. In: Photoassimilate Distribution in Plants and Crops, Zamski, E and Schaffer, A.A., eds., Marcel Dekker Publ, NYC, pp.63-96, 1996) which are under independent genetic control. Three separate *L. esculentum* genes coding for the large subunits and one gene for the small subunit have recently been cloned and sequenced (Chen, B.Y and Janes, H .1995, Plant Physiology 109.1498, Park, S W. and Chung, W I 1998 Gene 206 215-221) Much effort has been made

in order to identify sources of ADPGPPase genes in plants that may contribute to improving starch content, as for example in corn (Giroux, M.J. et al., Proc. Natl. Acad. Sci. USA 93:5824-5829), where site-specific mutation of the gene for the large subunit of ADPGPPase, using a transposable element *Ds* system, led to an insertion mutation of ADPGPPase which had decreased sensitivity to the ADPGPPase inhibitor, phosphate, as well as increased seed weight.

As regards to the use of wild species of *Lycopersicon* for the modification of carbohydrate metabolism in tomatoes, as described in US Patent *Application* 08/530,216, although the fructose to glucose ratio in *L. hirsutum* is high, the actual amount of fructose and glucose is very low. Recombination of the genetic trait of fructose to glucose ratio, together with the trait of high glucose and fructose levels from *L. esculentum* yielded the unobvious and desirable trait of high levels of hexose, together with the high ratio of fructose to glucose. However, *L. hirsutum* fruit accumulate only low amounts of starch, as compared to the cultivated *L. esculentum* (Miron and Schaffer, 1991, Plant Physiology 95:623-627). Similarly, other wild species of *Lycopersicon* also accumulate little starch (i.e., *L. chmieliewskii*, Yelle et al. 1988. Plant Physiology 87:737-740). Thus, the prior art has never expected or considered the use of wild tomatoes as a possible source of genetic variability for the increase in starch accumulation.

SUMMARY OF THE INVENTION

The present invention seeks to provide selection strategies for tomatoes with high starch content in the young fruit and subsequent high soluble solids in the mature fruit.

There is thus provided in accordance with a preferred embodiment of the present invention a method for controlling starch synthesis in tomatoes including providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes, and selecting individuals of the population that each contain an allele of a gene that increases starch synthesis, the gene originating from the *Lycopersicon* spp.

In accordance with a preferred embodiment of the present invention the step of selecting includes selecting individuals that each contain the allele of the gene that encodes for an enzyme that catalyzes a metabolic step in starch synthesis.

Further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting individuals that each contain the allele of the gene that encodes for a subunit of ADPGPPase.

Still further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting individuals that each contain the allele of the gene that

encodes for a *Lycopersicon hirsutum*-derived subunit of ADPGPPase.

Additionally in accordance with a preferred embodiment of the present invention the step of selecting includes selecting by using a molecular marker for the gene

In accordance with a preferred embodiment of the present invention the molecular marker includes step of selecting includes a *Lycopersicon hirsutum*-derived large subunit (LS1) of ADPGPPase.

Further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting by measuring activity of the enzyme in young fruit and selecting those young fruit with high activity of the enzyme.

Still further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting by measuring ADPGPPase activity of the young fruit, and selecting those young fruit with high ADPGPPase activity

In accordance with a preferred embodiment of the present invention the *Lycopersicon* spp. includes a *Lycopersicon* spp. of green-fruited *Eriopersicon* subgenus Preferably the *Lycopersicon* spp. includes *Lycopersicon hirsutum*.

There is also provided in accordance with a preferred embodiment of the present invention a method of producing genetically transformed plants which have elevated starch content, including the steps of inserting into the genome of a plant cell a recombinant double stranded DNA molecule including a selected promoter, a structural DNA sequence that causes the production of an RNA sequence which encodes the above described ADPGPPase LS1 protein, obtaining transformed plant cells, and regenerating from the transformed plant cells genetically transformed plants with elevated starch content.

In accordance with a preferred embodiment of the present invention the plant cell is selected from the group consisting of a tomato cell, a potato cell, a cell from a solanaceous plant, a legume cell, and a grain crop cell.

Further in accordance with a preferred embodiment of the present invention the promoter is selected from the group consisting of an immature fruit promoter, a tuber promoter, and a seed promoter.

Still further in accordance with a preferred embodiment of the present invention the step of regenerating includes regenerating genetically transformed plants with elevated starch content in an immature fruit

In accordance with a preferred embodiment of the present invention the step of regenerating includes regenerating genetically transformed plants with elevated starch content

in a tuber.

Further in accordance with a preferred embodiment of the present invention the step of regenerating includes regenerating genetically transformed plants with elevated starch content in a seed.

Still further in accordance with a preferred embodiment of the present invention the methods of the present invention also include the step of propagating the individuals of the population or the genetically transformed plants. The propagating may be by vegetative propagation or by seed, for example.

There are also provided in accordance with a preferred embodiment of the present invention a plant produced according to any of the methods of the present invention, a fruit produced by such a plant, and a seed which when grown yields such a plant.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be understood and appreciated more fully from the following detailed description, taken in conjunction with the drawing in which:

Figure 1 is a histogram of TSS (total soluble solids) values from individual plants of three BCF6 lines (95-929, 95-931 and 95-935), compared to a standard cultivar, M-82. Data from each plant is an average of TSS values from 5 individual fruit. Single plant selections from 95-929, 95-931 and 95-935 led to the BCF7 high starch breeding lines 900, 901 and 904, respectively.

In addition, the following tables are presented.

Table 1 shows the starch levels and activity of enzymes involved in the metabolism of sucrose to starch in young tomato fruit of the breeding lines 900, 901 and 904, compared to the standard cultivar, M-82. The * signifies statistical difference between each individual high starch line when compared to M-82 and does not indicate differences between the high starch lines. For the enzymes PGI (phosphoglucosisomerase), PGM (phosphoglucomutase) and UDPGPPase only one fruit was analyzed per line and since enzyme activity in all lines was relatively high and apparently in excess (as in Schaffer and Petreikov, 1997a) no significant differences were assumed. For the other assays, a minimum of 4 fruit from individual plants were assayed.

Table 2 shows the TSS values of mature fruit, and the starch levels of immature fruit of M-82, 904, the hybrid between them, a mix of 11 hybrids between 904 and 11 introgression lines (described in text), and a mix of the 11 parallel hybrids between M-82 and the same 11 introgression lines. At least two fruit from each of the individual hybrids were measured and

the average represents accordingly a minimum of 22 individual analyses At least three fruit from each of M-82, 904 and the hybrid between them were assayed.

Table 3 shows the enzyme activities of immature fruit pericarp of M-82, 904, the hybrid between them, a mix of 6 of the 11 hybrids between 904 and 11 introgression lines (described in text), and the parallel mix of 6 of the 11 hybrids between M-82 and the same introgression lines. For M-82, 904 and the hybrid between them, two fruit from individual plants were assayed.

Table 4 shows the nucleotide sequences of the forward and reverse primers used in the PCR analysis of the 3 large and 1 small subunits of ADPGPPase and the restriction endonucleases used to digest the PCR product in order to obtain the *L. hirsutum* specific allele.

Table 5 shows the activity levels of ADPGPPase of F2 plants from the cross of line 904 and M-82. The LS1 genotype of the plants was characterized at the seedling stage, as described further herein. ADPGPPase activity and starch levels are the averages from 4 fruit (8-13 gr.) from individual F2 plants TSS values are the average of a minimum of 5 fruit of each genotype.

Table 6 is the nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*

Table 7 is the derived amino acid sequence for ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

The following is one example of carrying out the present invention Plants of the *L. esculentum* breeding line 1630 (a Volcani Institute male sterile breeding line, used to simplify the production of the interspecific hybrid) were pollinated with pollen of the wild species *L. hirsutum* (LA1777). Hybrid F1 plants were grown and allowed to self pollinate, generating F2 seed. F2 seed were sown and about 350 plants were grown in a screenhouse and allowed to self pollinate.

Ripe fruit from each individual plant which produced fruit were individually analyzed for soluble solids (refractometrically). Only 25 of the interspecific F2 plants freely produced fruit. Pollen from one plant (F2-82) which was characterized by high soluble sugar level in the mature fruit (71 mg soluble sugar, composed of sucrose, glucose and fructose, per gram fresh weight of fruit) was used to pollinate a standard, industry type tomato (breeding line A701) for the production of the backcross-F1 (BC-F1) population. 100 BC-F1 plants were grown in the field and mature fruit of individual plants were analyzed for soluble solids, refractometrically,

as well as soluble sugars, as above. A pedigree, single seed descent selection program was carried out, selecting the plants with highest total soluble solids and soluble sugar levels. Each generation consisted of at least 100 plants. This selection technique was carried out for six generations, until the BC-F7 generation, leading to breeding lines with higher solids levels than the standard industry type cultivars.

Fig. 1 shows a series of histograms representing the BCF6 lines from which three BCF7 breeding lines were selected. The BCF6 95-929 had an average TSS value of 4.8 (11 plants, 5 fruits per plant), the BCF6 95-931 had an average TSS value of 5 7 (8 plants, 5 fruits per plant) and the BCF6 95-935 had an average TSS value of 6.1 (15 plants, 5 fruits per plant), as compared to the standard cultivar, M-82 which had an average TSS value of 3.5 (10 plants, 5 fruits per plant). The individual plant selection 95-929-6, which led to the BCF7 line 900, had a TSS of 5 5 with a plant yield of 9 1 kg fruit The individual plant selection 95-931-2, which led to the BCF7 line 901, had a TSS of 6.5 with a plant yield of 7 2 kg fruit The individual plant selection 95-935-5, which led to the BCF7 line 904, had a TSS of 6.6 with a plant yield of 4.7 kg fruit. The average plant yield of M-82 was 6 1 kg, based on an average of 6 plants.

In the BC-F7 generation immature fruit (approx. 15 days after anthesis) were measured for starch levels, as described in Schaffer and Petreikov (1997a). Lines 900, 901 and 904 were characterized by immature starch levels significantly higher than that of a standard industry type tomato cultivar, M-82 (Table 1). A comparative survey of enzymatic activities involved in sucrose to starch metabolism, as described in Schaffer and Petreikov (1997a), was performed on immature fruit of the two breeding lines and the standard, M-82 Typical results are presented in Table 1 and show that breeding line 900 is characterized by significantly higher levels of activity of the enzymes ADPGPPase and fructokinase while lines 901 and 904 are characterized by significantly higher activities of the enzyme ADPGPPase alone. Line 904 is characterized by the highest levels of the enzyme ADPGPPase among the lines we studied and was used for further study of the role of ADPGPPase in starch accumulation and TSS levels of tomato fruit.

The high starch line 904 was further hybridized with eleven independent tomato breeding lines. In parallel, the standard industry type tomato cultivar, M-82, was similarly hybridized with each of these eleven lines. The eleven lines used were from the *L. pennellii* introgression lines (ILS) These introgression lines are a set of purebred lines each containing a small chromosome segment of the wild green-fruited *Lycopersicon pennellii* in the background

of the cultivated *L. esculentum* cv M-82 (Eshed et al., 1992, Theor Appl. Genet., 83:1027-1034). These lines were developed from an initial interspecific cross between *L. pennellii* and *L. esculentum* cv M-82 The resulting F1 individuals were backcrossed to *L. esculentum* cv M-82 and selfed for several generations. During the process, chromosome segments of *L. pennellii* were selected for using restriction fragment length polymorphism probes covering the entire tomato genome. The introgression lines therefore provide a set of nearly-isogenic lines for segments of the wild-species genome and enable the association of yield traits with specific wild-species chromosome segments (Eshed Y and Zamir D. 1994. Theor Appl. Genet., 88:891-897) Eleven such introgression lines were used for this study. The assumption was that crossing the 904 high starch line with this broad spectrum of genotypes, and crossing in parallel M-82 with the same identical genotypes would supply us with a broad spectrum of genetic background in which the genetic effect of 904 could be discerned

Starch levels of the immature fruit, as well as soluble solids levels of the mature fruit, from the average of the eleven hybrids with line 904 were significantly higher than starch levels of immature fruit and soluble solids levels from mature fruit from the parallel hybrids with M-82 (Table 2). A number of these immature fruit, representing the high starch hybrids with 904 and the low starch hybrids with M-82 were subjected to a detailed enzymatic analysis of the enzymes involved in sucrose to starch metabolism in the immature tomato fruit (as described above). Table 3 shows that of the ten enzymes assayed, only ADPGPPase activity was significantly higher in the hybrids with the high starch line (904), compared to the hybrids with the M-82 line.

Table 1: Starch levels and enzyme activities of immature tomato fruit (approximately 15 DAA) for CV M-82 and three high starch breeding lines 900, 901 and 904.

	M-82	900	901	904
Starch (mg/gfw)	13.1	23.3 *	23.2 *	34 9 *
Enzymes (nmol/gfw/min)				
Invertase	15480	14690	18980	17870
Sucrose synthase	29570	31970	33260	27570
fructokinase	91	150 *	92	137
phosphoglucomutase	5760	6650	7830	7490
phosphoglucosisomerase	1950	2000	2870	2060
UDPglu PPase	15080	16760	17250	14760
ADPglu PPase	40	142 *	84 *	268 *

*Indicates statistical significance ($P < 0.05$) of each individual high starch line as compared to M-82.

Table 2: Starch content of immature fruit (approx. 15 days after anthesis) and °Brix (TSS) values of mature fruit of line 904, M-82, the hybrid between them, the mix of 11 hybrids between M-82 and 11 introgression lines (ILS) and the mix of 11 hybrids between 904 and the same 11 ILS.

Genotype	Starch mg/gfw	°Brix
M-82	23 b	4.1 b
904	58 a	8.1 a
M-82 x 904	46 a	7.1 a
M-82 x ILS	25 b	5.3 b
904 x ILS	44 a	7.5 a

Letters signify statistical significance at $P < 0.05$

Table 3: Activities of enzymes in the sucrose to starch metabolic pathway in immature tomato fruit.

	Activity (nmol/gfw/min)		
Enzyme	904 x ILS	M-82 x ILS	Ratio
Invertase	520	620	0.83
Sucrose synthase	710	560	1.27
fructokinase	225	219	1.03
glucokinase	23	25	0.94
phosphoglucomutase	6900	5340	1.31
phosphoglucoisomerase	3160	2630	1.21
UDPglu PPase	8490	7130	1.19
ADPglu PPase	190	56	3.67*
starch synthase, sol.	48	38	1.26
starch synthase, insol.	5	5	0.93

* statistical significance at $P < 0.05$

To further study the genetic trait for high ADPGPPase activity in immature fruit, specific DNA primers for the genes for the four ADPGPPase subunits (Chen and Janes, 1997 and Park and Cheung, 1998) were devised which could distinguish between the *L. hirsutum* derived gene and the *L. esculentum* derived gene, as described in the following paragraph

PCR analysis of ADPGPPase subunits

Amplification reactions of the ADPGPPase subunits (25 µl final volume) contained 10 ng template DNA, 25 mM TAPS (pH=9 3 at 25°C), 50 mM KCl, 2.5 mM $MgCl_2$, 1 mM (mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 10 ng of each of the 2 primers (forward and reverse primers, see Table 4), and 1 unit of thermostable Taq DNA polymerase (SuperNova Taq polymerase, Madi Ltd., Rishon Le Zion, Israel). Reactions were carried out in an automated thermocycler (MJ Research Inc., Watertown, Massachusetts, USA). Initial incubation was at 94°C for 1 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and polymerization at 72°C for 1 min and 45 sec. Final polymerization at 72°C was carried out for

7 min after cycles were completed. 10 µl of the amplification products were digested with 15 units of the restriction endonuclease found to generate the *L. hirsutum* specific alleles (Table 4). Digestions were carried out according to the manufacturer recommendations (New England Biolabs Inc., Beverly, MA, USA). The digestion products were visualized by electrophoresis in 1.2% agarose gel and detected by staining with ethidum bromide.

Line 904 was shown to carry the *L. hirsutum* gene for large subunit 1 (LS1) while the other subunits of ADPGPPase in line 904 were shown to be derived from the *L. esculentum*.

In order to show that the *L. hirsutum* derived LS1 was correlated with increased ADPGPPase activity and increased starch level in the immature fruit, an F2 population of 64 plants of the cross between the high starch line 904 and the standard line M-82 was grown. The plants were genotypically typed at the first true leaf stage to determine whether they were homozygous for the *L. hirsutum* ADPGPPase LS1 allele (HH), homozygous for the *L. esculentum* allele (EE) or heterozygous (HE) containing both alleles The 64 F2 plants segregated for the LS1 in a ratio of 16:31:17, as expected for a single locus. Immature fruit from a minimum of 4 of each of the determined F2 genotypes were assayed for starch levels and for ADPGPPase activity. Results are presented in Table 5 and clearly show that the *L. hirsutum* allele for ADPGPPase LS1, as characterized by the specific PCR primers described, is associated with increased ADPGPPase activity in the immature fruit. Furthermore, the TSS values of the mature fruit was similarly influenced by the genotype of the LS1 gene.

Table 4. Forward and reverse primers used in the PCR analysis of the 3 large and 1 small subunits of ADPGPPase and the restriction endonuclease used to digest the PCR product in order to obtain the *L. Hirsutum* specific allele

ADPGPPase Subunit	Forward primer	Reverse primer	Restriction endonuclease
Large (LS1)	GTTCATTTGGGGA GAGTGAGCAC	GGGCAGCAGAAT TGTACTGTGTC	Hinf I
Large (LS2)	CTATTGGTGGTTG TTACCGGGT	CACTGTTCCAATA TCCTCCCAG	Hinf I
Large (LS3)	GCATATTGCTCGT GCGTACAAC	CTTTTCGCTGAAG GACATGACC	-
Small	TTTCGTCTTCTCA TCTCGCCGGA	GGCGATTTAGAG AGGCAGAGTTG	RsaI

Table 5: Effect of genotype of LS1 on ADPGPPase activity and starch levels in immature fruit and TSS in mature fruit. ADPGPPase activity and starch levels are the averages from 4 fruit (8-13 gr.) from individual F2 plants. TSS values are the average of a minimum of 5 fruit of each genotype.

Genotype	ADPGPPase	Starch	TSS
EE	104 c	16.4 b	5.3 b
EH	306 b	25.2 ab	5.9 ab
HH	450 a	37.3 a	6.3 a

Letters signify statistical difference at $P < 0.05$

Sequencing of the gene encoding ADPGPPase large subunit (LS1) from *L. hirsutum*.

Total RNA was extracted from young fruits (3 grams in weight) of an individual plant homozygous for the ADPGPPase large subunit (LS1). The RNA extraction was carried out using the TRIzol reagent system (GibcoBRL life technologies, Gaithersburg, MD, USA). The total RNA was used as template for first strand cDNA synthesis using the Superscript preamplification system (GibcoBRL life technologies, Gaithersburg, MD, USA) The cDNA prepared was used as template in a PCR reaction to amplify the gene encoding ADPGPPase large subunit (LS). The DNA fragments containing the ADPGPPase large subunit (LS) were excised from an agarose gel and purified using the GENECLEAN II kit (BIO 101 inc., La Jolla CA, USA). The PCR bands were then cloned into an pGEM-T Easy vector using the pGEM-T and pGEM-T Easy Vector Systems according to the manufacturer recommendations (Promega corporation, Madison, WI, USA). The DNA clones were sequenced using an automated sequencer (Applied Biosystems, Foster City, CA, USA).

Table 6 is the nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L.hirsutum* Table 7 is the derived amino acid sequence for ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*.

Table 6: Nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*

```
1    ATGAAATCGA CGGTTCATTT GGGGAGAGTG AGCACTGGTG CTTTAACAA
51   TGGAGAGAAG GAGATTTTTG GGGAGAAGAT GAGAGGGAGT TTGAACAACA
101  ATCTCAGGAT TAATCAGTTG TCGAAAAGTT TGAAACTTGA GAAGAAGGAG
151  AAGAAGATTA AACCTGGGGT TGCTTACTCT GTGATCACTA CTGAAAATGA
201  CACAGAGACT GTGTTCGTAG ATATGCCACG TCTTGAGAGA CGCCGGGCAA
251  ATCCCAAGGA TGTGGCTGCA GTCATATTAG GAGGAGGCGA AGGGACCAAG
301  TTATTCCCAC TTACAAGTAG AACTGCAACC CCTGCTGTTC CGGTTGGAGG
351  ATGCTACAGG CTCATAGACA TCCCGATGAG CAACTGTATC AACAGTGCTA
401  TTAACAAGAT TTTTGTGCTG ACACAGTACA ATTCTGCTGC CCTGAATCGT
451  CACATTGCTC GAACGTATTT TGGCAATGGT GTGAGCTTTG GAGATGGATT
501  TGTCGAGGTA CTAGCTGCAA CTCAGACACC TGGGGAAGCA GGAAAAAAAT
551  GGTTTCAAGG AACAGCAGAT GCTGTCAGAA AATTTATATG GGTTTTTGAG
601  GACGCTAAGA ACAAGAATAT TGAAAATATC CTTGTATTAT CTGGGGATCA
651  TCTTTATAGG ATGGATTATA TGGAGTTGGT GCAGAACCAT ATTGACAGAA
701  ATGCTGATAT TACTCTTTCA TGTGCACCAG CTGAGGACAG CCGAGCATCA
751  GATTTTGGGC TGGTCAAGAT TGACAGCAGA GGCAGAGTTG TCCAGTTTGC
801  TGAAAAACCA AAAGGTTTTG AGCTTAAAGC AATGCAAGTA GATACTACTC
851  TTGTTGGATT ATCTCCACAA GATGCGAAGA AATCCCCTTA TATTGCTTCA
901  ATGGGAGTTT ATGTTTTCAA GACAGATGTA TTGCTGAAGC TCTTGAAATG
951  GAGCTACCCC ACTTCTAATG ATTTTGGCTC TGAAATTATA CCAGCAGCTA
1001 TTGATGATTA CAATGTCCAA GCATACATTT TCAAAGACTA TTGGGAGGAC
1051 ATTGGAACAA TTAAATCTTT CTATAATGCT AGCTTGGCGC TCACACAAGA
1101 GTTTCCAGAG TTCCAATTTT ATGATCCAAA AACACCTTTT TACACATCTC
1151 CTAGGTTCCT TCCACCAACC AAGATAGACA ATTGCAAGAT TAAGGATGCC
1201 ATAATTTCTC ATGGATGTTT CTTGCGAGAT TGCTCTGTGG AACACTCCAT
1251 AGTGGGTGAA AGATCACGCT TAGACTGTGG TGTTGAACTG AAGGATACTT
1301 TCATGATGGG AGCAGACTAC TACCAAACAG AATCTGAGAT TGCCTCCCTG
1351 TTAGCAGAGG GGAAAGTACC GATTGGGATT GGGGAAAATA CAAAAATAAG
1401 GAAATGTATC ATTGACAAGA ACGCAAAGAT AGGAAAAAAT GTTTCAATCA
1451 TTAATAAAGA TGGTGTTCAA GAGGCAGACC GACCAGAGGA AGGATTCTAC
1501 ATACGATCAG GGATAACCAT TATATCAGAG AAAGCCACAA TTAGAGATGG
1551 AACAGTTATA TGA
```

Table 7: Derived amino acid sequence for ADPGPPase LS1 from *L. hirsutum*

```
MKSTVHLGRVSTGGFNNGEKEIFGEKMRGSLNNNLRINQL
SKSLKLEKKEKKIKPGVAYSVITTENDTETVFVDMPRLERRRAN
PKDVAAVILGGGEGTKLFPLTSRTATPAVPVGGCYRLIDIPMSNC
INSAINKIFVLTQYNSAALNRHIARTYFGNGVSFGDGFVEVLAAT
QTPGEAGKKWFQGTADAVRKFIWVFEDAKNKNIENILVLSGDHL
YRMDYMELVQNHIDRNADITLSCAPAEDSRASDFGLVKIDSRGR
VVQFAEKPKGFELKAMQVDTTLVGLSPQDAKKSPYIASMGVYV
FKTDVLLKLLKWSYPTSNDFGSEIIPAAIDDYNVQAYIFKDYWED
IGTIKSFYNASLALTQEFPEFQFYDPKTPFYTSPRFLPPTKIDNCKI
KDAIISHGCFLRDCSVEHSIVGERSRLDCGVELKDTFMMGADYY
QTESEIASLLAEGKVPIGIGENTKIRKCIIDKNAKIGKNVSIINKDG
```

VQEADRPEEGFYIRSGITIISEKATIRDGTVI

In the foregoing example, the large subunit 1 of ADPGPPase was shown to increase starch level. Although not specifically tested, it is reasonable to assume that the present invention can also be carried out by transferring the *L. hirsutum* genes for any of the other 3 subunits of the enzyme, using the specific PCR markers developed for each of these genes, as they may also increase starch. In addition, transfer of ADPGPPase genes from other wild tomato species, other than *L. hirsutum*, may also increase starch in crosses with *L. esculentum*. Additionally, transfer of genes for other enzymes of starch synthesis from wild species, such as fructokinase and sucrose synthase for which the gene sequences from *L. esculentum* are known, may also increase starch levels.

Those skilled in the art will recognize that the described gene can be used to genetically transform plants to increase starch content. Plants that can genetically be transformed to have increased starch content include a large range of agriculturally important crops, such as but not limited to, potato, tomato, corn, wheat, cotton, banana, soybean, pea and rice The plant transformation technology, including methods of transformation, such as the use of *Agrobacterium tumefaciens*, and methods of developing constructs, including the use of tissue specific promoters is well established and has recently been reviewed by Christou, P. ("Transformation technology", Trends in Plant Science, 1:423-431) There are presently available numerous promoters, including the constitutive promoters (CaMV) 35S and the maize ubiquitin promoter In addition, there are, for example, organ/tissue specific promoters, for expression in seeds, tubers, immature fruit, mature fruit, pollen, roots and other organs

The above examples are provided to better elucidate the practice of the present invention and should not be interpreted in any way to limit the scope of the present invention. Those skilled in the art will recognize that various modifications can be made to the methods described herein while not departing from the spirit and scope of the present invention.

CLAIMS

What is claimed is:

1. A method for controlling starch synthesis in tomatoes comprising:

providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes; and

selecting individuals of said population that each contain an allele of a gene that increases starch synthesis, said gene originating from said *Lycopersicon* spp.

2. The method according to claim 1 wherein said step of selecting comprises selecting individuals that each contain the allele of the gene that encodes for an enzyme that catalyzes a metabolic step in starch synthesis.

3. The method according to claim 1 wherein said step of selecting comprises selecting individuals that each contain the allele of the gene that encodes for a subunit of ADPGPPase.

4. The method according to claim 1 wherein said step of selecting comprises selecting individuals that each contain the allele of the gene that encodes for a *Lycopersicon hirsutum*-derived subunit of ADPGPPase

5. The method according to claim 1 wherein said step of selecting comprises selecting by using a molecular marker for said gene

6. The method according to claim 5 wherein said molecular marker comprises step of selecting comprises a *Lycopersicon hirsutum*-derived large subunit (LS1) of ADPGPPase.

7. The method according to claim 2 wherein said step of selecting comprises selecting by measuring activity of said enzyme in young fruit and selecting those young fruit with high activity of said enzyme

8. The method according to claim 2 wherein said step of selecting comprises selecting by measuring ADPGPPase activity of said young fruit, and selecting those young fruit with high ADPGPPase activity.

9. The method according to claim 1 wherein said *Lycopersicon* spp. comprises a *Lycopersicon* spp. of green-fruited *Eriopersicon* subgenus.

10. The method according to claim 1 wherein said *Lycopersicon* spp. comprises *Lycopersicon hirsutum*

11. A method of producing genetically transformed plants which have elevated starch content, comprising the steps of:

a) inserting into the genome of a plant cell a recombinant double stranded DNA

molecule comprising

(i) a selected promoter

(ii) a structural DNA sequence that causes the production of an RNA sequence which encodes the above described ADPGPPase LS1 protein

b) obtaining transformed plant cells

c) regenerating from the transformed plant cells genetically transformed plants with elevated starch content.

12. The method according to claim 11 wherein said plant cell is selected from the group consisting of a tomato cell, a potato cell, a cell from a solanaceous plant, a legume cell, and a grain crop cell.

13. The method according to claim 11 wherein said promoter is selected from the group consisting of an immature fruit promoter, a tuber promoter, and a seed promoter

14. The method according to claim 11 wherein said step of regenerating comprises regenerating genetically transformed plants with elevated starch content in an immature fruit.

15. The method according to claim 11 wherein said step of regenerating comprises regenerating genetically transformed plants with elevated starch content in a tuber.

16. The method according to claim 11 wherein said step of regenerating comprises regenerating genetically transformed plants with elevated starch content in a seed.

17. A method according to claim 1 and additionally comprising the step of propagating said individuals of said population.

18. A method according to claim 17 wherein the step of propagating includes the step of vegetative propagation.

19. A method according to claim 17 wherein the step of propagating includes the step of propagation by seed.

20. A method according to claim 11 and additionally comprising the step of propagating said genetically transformed plants.

21. A method according to claim 20 wherein the step of propagating includes the step of vegetative propagation.

22. A method according to claim 20 wherein the step of propagating includes the step of propagation by seed.

23. A plant produced according to the method of claim 1.

24. A plant produced according to the method of claim 11.

25. A fruit produced by a plant in accordance with claim 23.

26. A fruit produced by a plant in accordance with claim 24

27. A seed which when grown yields a plant in accordance with claim 23

28. A seed which when grown yields a plant in accordance with claim 24

29. A gene that controls sucrose-starch metabolism comprising a nucleotide sequence as follows:

1 ATGAAATCGA CGGTTCATTT GGGGAGAGTG AGCACTGGTG CTTTAACAA
51 TGGAGAGAAG GAGATTTTTG GGGAGAAGAT GAGAGGGAGT TTGAACAACA
101 ATCTCAGGAT TAATCAGTTG TCGAAAAGTT TGAAACTTGA GAAGAAGGAG
151 AAGAAGATTA AACCTGGGGT TGCTTACTCT GTGATCACTA CTGAAAATGA
201 CACAGAGACT GTGTTCGTAG ATATGCCACG TCTTGAGAGA CGCCGGGCAA
251 ATCCCAAGGA TGTGGCTGCA GTCATATTAG GAGGAGGCGA AGGGACCAAG
301 TTATTCCCAC TTACAAGTAG AACTGCAACC CCTGCTGTTC CGGTTGGAGG
351 ATGCTACAGG CTCATAGACA TCCCGATGAG CAACTGTATC AACAGTGCTA
401 TTAACAAGAT TTTTGTGCTG ACACAGTACA ATTCTGCTGC CCTGAATCGT
451 CACATTGCTC GAACGTATTT TGGCAATGGT GTGAGCTTTG GAGATGGATT
501 TGTCGAGGTA CTAGCTGCAA CTCAGACACC TGGGGAAGCA GGAAAAAAAT
551 GGTTTCAAGG AACAGCAGAT GCTGTCAGAA AATTTATATG GGTTTTTGAG
601 GACGCTAAGA ACAAGAATAT TGAAAATATC CTTGTATTAT CTGGGGATCA
651 TCTTTATAGG ATGGATTATA TGGAGTTGGT GCAGAACCAT ATTGACAGAA
701 ATGCTGATAT TACTCTTTCA TGTGCACCAG CTGAGGACAG CCGAGCATCA
751 GATTTTGGGC TGGTCAAGAT TGACAGCAGA GGCAGAGTTG TCCAGTTTGC
801 TGAAAAACCA AAAGGTTTTG AGCTTAAAGC AATGCAAGTA GATACTACTC
851 TTGTTGGATT ATCTCCACAA GATGCGAAGA AATCCCCTTA TATTGCTTCA
901 ATGGGAGTTT ATGTTTTCAA GACAGATGTA TTGCTGAAGC TCTTGAAATG
951 GAGCTACCCC ACTTCTAATG ATTTTGGCTC TGAAATTATA CCAGCAGCTA
1001 TTGATGATTA CAATGTCCAA GCATACATTT TCAAAGACTA TTGGGAGGAC
1051 ATTGGAACAA TTAAATCTTT CTATAATGCT AGCTTGGCGC TCACACAAGA
1101 GTTTCCAGAG TTCCAATTTT ATGATCCAAA AACACCTTTT TACACATCTC
1151 CTAGGTTCCT TCCACCAACC AAGATAGACA ATTGCAAGAT TAAGGATGCC
1201 ATAATTTCTC ATGGATGTTT CTTGCGAGAT TGCTCTGTGG AACACTCCAT
1251 AGTGGGTGAA AGATCACGCT TAGACTGTGG TGTTGAACTG AAGGATACTT
1301 TCATGATGGG AGCAGACTAC TACCAAACAG AATCTGAGAT TGCCTCCCTG
1351 TTAGCAGAGG GGAAAGTACC GATTGGGATT GGGGAAAATA CAAAAATAAG
1401 GAAATGTATC ATTGACAAGA ACGCAAAGAT AGGAAAAAAT GTTTCAATCA
1451 TTAATAAAGA TGGTGTTCAA GAGGCAGACC GACCAGAGGA AGGATTCTAC
1501 ATACGATCAG GGATAACCAT TATATCAGAG AAAGCCACAA TTAGAGATGG
1551 AACAGTTATA TGA

30. A protein that controls sucrose-starch metabolism comprising a derived amino acid sequence as follows:

MKSTVHLGRVSTGGFNNGEKEIFGEKMRGSLNNNLRINQL
SKSLKLEKKEKKIKPGVAYSVITTENDTETVFVDMPRLERRRAN
PKDVAAVILGGGEGTKLFPLTSRTATPAVPVGGCYRLIDIPMSNC
INSAINKIFVLTQYNSAALNRHIARTYFGNGVSFGDGFVEVLAAT
QTPGEAGKKWFQGTADAVRKFIWVFEDAKNKNIENILVLSGDHL
YRMDYMELVQNHIDRNADITLSCAPAEDSRASDFGLVKIDSRGR

VVQFAEKPKGFELKAMQVDTTLVGLSPQDAKKSPYIASMGVYV
FKTDVLLKLLKWSYPTSNDFGSEIIPAAIDDYNVQAYIFKDYWED
IGTIKSFYNASLALTQEFPEFQFYDPKTPFYTSPRFLPPTKIDNCKI
KDAIISHGCFLRDCSVEHSIVGERSRLDCGVELKDTFMMGADYY
QTESEIASLLAEGKVPIGIGENTKIRKCIIDKNAKIGKNVSIINKDG
VQEADRPEEGFYIRSGITIISEKATIRDGTVI

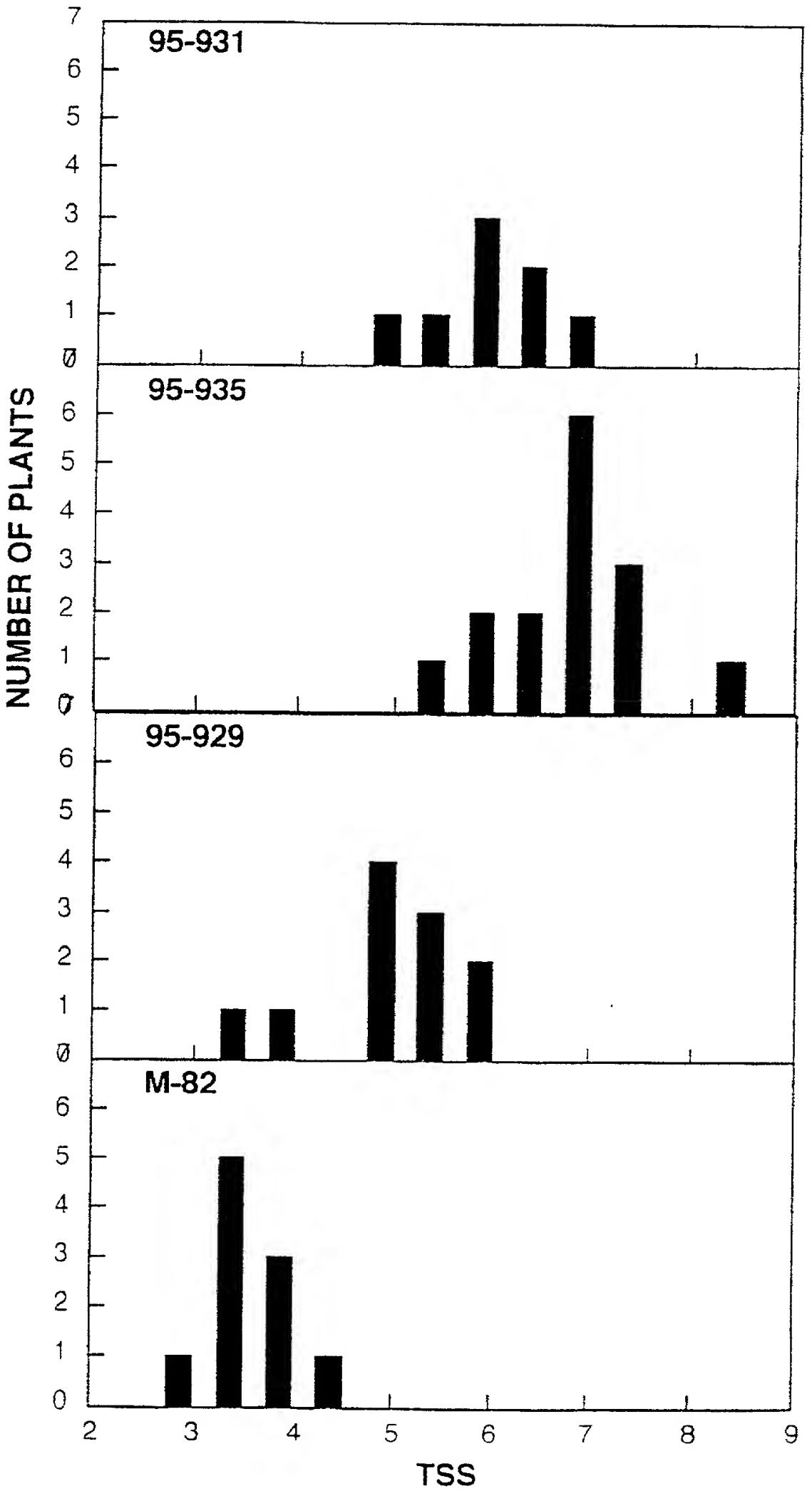

FIG. 1

26 MAR 2001

Practitioner's Docket No. _____ *PATENT* #3

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

[ ] original.
[ ] design.
[ ] supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

[X] national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

NOTE: See 37 CFR 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

[ ] divisional.
[ ] continuation.

NOTE: Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 CFR 1.53(b) (application filing requirements-nonprovisional application).

[ ] continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

CONTROLLING STARCH SYNTHESIS

SPECIFICATION IDENTIFICATION

The specification of which:

(complete (a), (b), or (c))

(a) [ ] is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) [X] was filed on Jan. 19, 2001, as [X] Serial No. 09/744,085 on
[ ] _____ and was amended on _____ *(if applicable).*

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and application number (consisting of the series code and the serial number; e.g.,08/123,456);

"(2) name of inventor(s), serial number and filing date;

"(3) name of inventor(s) and attorney docket number which was on the specification as filed;

"(4) name of inventor(s), title which was on the specification as filed and filing date;

"(5) name of inventor(s), title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or

"(6) name of inventor(s), title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number; e.g.,08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration."

Notice of July 13, 1995 (1177 O.G. 60).

(c) [ ] was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____ *(if any)*.

SUPPLEMENTAL DECLARATION (37 CFR 1.67(b))

(complete the following where a supplemental declaration is being submitted)

[ ] I hereby declare that the subject matter of the

[ ] attached amendment
[ ] amendment filed on _____.

was part of my/our invention and was invented before the filing date of the original application, above identified, for such invention.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56,

(also check the following items, if desired)

[ ] and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

[ ] in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119(a)-(d))

NOTE: *"The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by § 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. § 119(b) must be filed in the case of an interference (§ 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in § 1.17(i). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate."* 37 CFR 1.55(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) [ ] no such applications have been filed.

(e) [X] such applications have been filed as follows

NOTE: *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING DAY, MONTH, YEAR	PRIORITY CLAIMED UNDER 35 USC 119
PCT	PCT/IL99/00396	19, July, 1999	[X]YES [ ]NO
Israel	125425	20, July, 1998	[X]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
____/_____	_____
____/_____	_____
____/_____	_____

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S) UNDER 35 U.S.C. § 120

[ ] The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

_____

_____

_____

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

JOSEPH H. HANDELMAN, 26179

JOHN RICHARDS, 31053

RICHARD J. STREIT, 25765

PETER D. GALLOWAY, 27885

IAN C. BAILLIE, 24090

THOMAS F. PETERSON, 24790

RICHARD P. BERG, 28145

JULIAN H. COHEN, 20302

WILLIAM R. EVANS, 25858

JANET I. CORD, 33778

CLIFFORD J. MASS, 30086

(Check the following item, if applicable)

[ ] Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO

Ladas & Parry
26 West 61st Street
New York, N.Y. 10023

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

1-00

Full name of sole or first inventor

Arthur (Given Name) (Middle Initial or Name) SCHAEFER (Family (Or Last Name)

Inventor's signature X

Date X 25.3.01 **Country of Citizenship** Israel

Residence Hashmonaim 73127, Israel IL X

Post Office Address 16 Hazavit Street, Hashmonaim 73127, Israel

2-00

Full name of second joint inventor, if any

Ilan (Given Name) (Middle Initial or Name) LEVIN (Family (Or Last Name)

Inventor's signature X Ilan Levin

Date X 25.3.01 **Country of Citizenship** Israel

Residence Mazkeret Batya, 76804, Israel

Post Office Address 8 Eshel Street, Mazkeret Batya 76804, Israel

3-00

Full name of third joint inventor, if any

Marina (Given Name) (Middle Initial or Name) PETREIKOV (Family (Or Last Name)

Inventor's signature X MPetr

Date X 25.03.01 **Country of Citizenship** Israel

Residence 55/22 Bernstein Street, Rishon LeZion 75000, Israel IL X

Post Office Address 55/22 Bernstein Street, Rishon LeZion 75000, Israel

Attorney's Docket No. [redacted]

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

4-00

Full name of fourth joint inventor, if any

Moshe (GIVEN NAME) [redacted] (MIDDLE INITIAL OR NAME) BAR (FAMILY OR LAST NAME)

Inventor's signature X Moshe Bar

Date X 25/3/01 Country of Citizenship Israel

Residence Rishon LeZion 75246, Israel LLX

Post Office Address 10 NahaliSorag Street, Rishon LeZion 75246, Israel

[redacted]

Full name of fifth joint inventor, if any

[redacted] (GIVEN NAME) [redacted] (MIDDLE INITIAL OR NAME) [redacted] (FAMILY OR LAST NAME)

Inventor's signature [redacted]

Date [redacted] Country of Citizenship [redacted]

Residence [redacted]

Post Office Address [redacted]

[redacted]

Full name of sixth joint inventor, if any

[redacted] (GIVEN NAME) [redacted] (MIDDLE INITIAL OR NAME) [redacted] (FAMILY OR LAST NAME)

Inventor's signature [redacted]

Date [redacted] Country of Citizenship [redacted]

Residence [redacted]

Post Office Address [redacted]

(Added Page to Combined Declaration and Power of Attorney for Signature by Fourth and Subsequent Inventors [1.2])

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

[X] Signature for fourth and subsequent joint inventors. *Number of pages added* 1

* * *

[ ] **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* ___

* * *

[ ] **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

* * *

[ ] Added page for signature by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

* * *

[ ] Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

[ ] Number of pages added _____

* * *

[ ] Authorization of practitioner(s) to accept and follow instructions from representative.

(If no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

[ ] This declaration ends with this page.